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# The effects of partial replacement of fish meal by vegetable protein sources in the diet of rainbow trout (*Onchorynchus mykiss*) on post mortem spoilage of fillets

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**Abstract**

Five test diets were formulated with decreasing levels of fish meal (up to 50%) replaced by alternative protein sources. Rainbow trout were fed the experimental diets for 12 weeks.The effects of feed ingredients on spoilage of *Oncorhynchus mykiss* in ice and under MAP/ice (40% $CO_2$, 30% $N_2$ and 30% $O_2$) were investigated in terms of sensory, chemical and microbiological analyses. The results showed that the trout in MAP/ice was rejected at 14 days, after sensory analysis, due to excessive drip, whereas trout in ice were found to be acceptable even after 14 days of storage. However, cooked trout fillets, under both storage conditions, were rejected at 17 days. Fish in ice produced higher *K* values and higher concentrations of biogenic amines during the storage period of 17 days than the fish in MAP/ice. Bacteria grew more quickly in rainbow trout kept in ice than in MAP/ice. MAP/ice storage extended the shelf life of rainbow trout by approximately 2 days compared to ice storage alone in terms of microbiological analyses.


*Keywords:* Rainbow trout; Alternative protein sources; MAP; Storage life

## 1. Introduction

Intensive aquaculture continues to expand, which requires high quality protein sources. Fish meal is major and increasingly expensive component of salmon and trout feeds (Carter & Hauler, 2000) since it has high levels of digestible protein and energy, excellent amino acid and fatty acid profiles (Mwachireya, Beames, Higgs, & Dosanjh, 1999; Rumsey, 1993) compared to plant protein sources. Considerable attention have been given to the potential use of soybean meals in feeds for salmonids (Adelizi et al., 1998; Carter, Houlihan, Buchanan, & Mitchell, 1994; Hardy, 1995, 1996; Olivia-Teles, Gouveria, Gomes, & Rema, 1994; Olli & Krogdahl, 1995; Refstie, Storebakken, & Roem, 1998; Watanabe & Pongmaneerat, 1993; Watanabe, Pongmaneerat, Sato, & Takeucki, 1993). However, less attention has been paid to other plant protein sources, which are associated with low protein levels and the presence of antinutritional factors (Francis, Makkar, & Becker, 2001).

Sensory characteristics have major effects on the acceptance and market value of the products. Before selection of ingredients for replacement of fish meal is recommended, the effects of ingredients on the sensory and flavour of the fish should be known. The inclusion of vegetable proteins in the diets of salmonids did not influence the sensory characteristics of the fish (Bjerkeng et al., 1997; Skonberg, Hardy, Barrows, & Dong, 1998;

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Smith, Kincaid, Regenstein, & Rumsey, 1988; Yurkowski, Bailey, Evans, Tabachek, & Ayles, 1978). However, Kaushik et al. (1995) reported subtle sensory effects when rainbow trout were fed a diet containing a soy protein.

Modified atmosphere packing (MAP), along with refrigeration, has the ability to extend the shelf life of food (Banks, Nickelson, & Finne, 1980; Davis, 1993; Farber, 1991) by inhibiting psychrotopic, aerobic and Gram negative bacteria (Banks et al., 1980; Brown et al., 1980; Finne, 1982). The effects of MAP on seafood have been extensively reviewed (Farber, 1991; Reddy, Armstrong, Rhodehamel, & Kauter, 1992; Sivertsvik, Jeksrund, & Rosnes, 2002; Stammen, Gerdes, & Caporaso, 1990). Although there are several studies related to MAP storage of rainbow trout (Barnett, Conrad, & Nelson, 1987; Cann et al., 1984; Randell, Ahvenainen, & Hattula, 1995), less information is available on the effects of MAP on the sensory, chemical and microbiological quality of rainbow trout fed with different vegetable protein sources. Therefore, the objectives of the current study are to investigate the effects of feed ingredients on spoilage of rainbow trout stored in ice and MAP/ice (40% $CO_2$, 30% $N_2$ and 30% $O_2$) in terms of sensory, chemical and microbiological analyses.

## 2. Materials and methods

### 2.1. Experimental diets and fish

Five test nitrogenous diets of approximately equal gross energy (2.1 MJ/100 g) were formulated with decreasing levels of fish meal, using alternative protein sources. In the five experimental diets, fish meal was gradually replaced by the plant protein sources (maize gluten, canola meal, pea protein, full fat soya, soybean meal, soybean concentrate and wheat gluten) (Table 1). The combination of these protein meals was used to replace 13%, 26%, 38% and 50% of the fish meal in the control diet for diets 2, 3, 4 and 5, respectively. The diets were formulated to contain almost the same levels of protein (45%) and lipid (22 %). All diets containing plant protein sources were also supplemented with lysine and methionine. Rainbow trout (55 g) were fed experimental diets for 12 weeks, reaching approximately 200 g. Duplicate tanks were used for each treatment and two feeding trials were conducted. Each time, three diets (including the control diet) were evaluated. Diets 3 and 5 were used for trial one while diets 2 and 4 were used for trial 2.

Table 1
Diets formulation

| Ingredients inclusion (%) | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 |
|---|---|---|---|---|---|
| LT94 Fish meal | 56.5 | 49.1 | 42 | 35 | 28 |
| Wheat | 14 | 10.3 | 10 | 10 | 10 |
| Full fat soya | 10 | 10 | 10 | 10 | 10 |
| Maize gluten | 6.2 | 10 | 10 | 10 | 10 |
| Canola meal | | 3.8 | 2.2 | | |
| Pea protein | | | 3.9 | 3.7 | 5 |
| Soybean meal | | | 1.5 | | |
| Soybean concentrate | | | | 8.8 | 10 |
| Wheat gluten | | | | | 2.4 |
| L-Lysine | | 1 | 1.6 | 2 | 3.2 |
| L-Methionine | | 0.1 | 0.2 | 0.4 | 0.5 |
| Fish oil | 14.9 | 15 | 15.4 | 16.9 | 17.7 |
| Feed stimulant | 1 | 1 | 1 | 1 | 1 |
| Vitamins and minerals[a] | 1 | 1 | 1 | 1 | 1 |
| Dicalcium phosphate | 1.9 | 1.8 | 0.5 | 0.5 | 0.5 |
| Astaxanthin mix | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |

[a] According to NRC (1993).

### 2.2. Packaging and storage of rainbow trout

After fish were harvested at the end of feeding trials, they were gutted and then divided into two lots. One lot was iced in boxes and the another lot was placed in nylon–polyethylene pouches (30 × 35 cm) and gas-packed in a Multivac model A 300 vacuum-packaging machine (Bury, Lancs., UK). Three fish were packed into a pouch. The gas composition was 40% $CO_2$, 30% $N_2$ and 30% $O_2$. The final gas/sample ratio, in all pouches, was about 3:1 (v/w) for MAP conditions. MAP packs were put into boxes containing ice to provide the same storage temperature. All samples were then stored in a chill room (22 °C). Three fish were taken for chemical and microbiological analysis from the two different storage conditions (ice and MAP/ice) every three or four days throughout the storage period (17 days). Dorsal parts of fish were used for analysis.

### 2.3. Sensory analysis

For sensory analysis, one fish from each of the two storage conditions was taken at intervals for each treatment. Each assessment was carried out by a minimum of 6-trained assessors. Sensory analysis was done using the Tasmanian Food Research Unit scheme (Branch & Vail, 1985) with minor modifications for gutted rainbow trout stored in ice and MAP/ice (Table 2). Each panellist was given up to four simple descriptors, which scored from 0 to a maximum of 3 where 0 represented good quality and higher score indicated poor quality. The scores for the separate characteristics were summed to give an overall sensory score. This system gives a 0 score for very fresh fish whereas higher values are obtained as fish deteriorate. Panellists were also asked to state whether or not the fish were acceptable. This was used to determine the shelf life of the fish. The freshness of cooked fish (odour flavour and texture) was assessed according to Torry Scheme (Howgate, 1982) with minor modifications for rainbow trout. A scale from 10 to $\leqslant 3$ was used to evaluate fish. Ten represents very fresh fish while $\leqslant 3$

Table 2
Modified Tasmanian food research unit freshness assessment scheme

| Score | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| *General* | | | | |
| Appearance | Very Bright | Bright | Slightly dull | Dull |
| Skin | Firm | Soft | | |
| Slime | Absent | Slightly slimy | Slimy | Very Slimy |
| Stiffness | Pre-rigor | Rigor | Post-rigor | |
| *Eyes* | | | | |
| Clarity | Clear | Slightly cloudy | Cloudy | |
| Shape | Normal | Slightly sunken | Sunken | |
| Iris | Visible | Not visible | | |
| Blood | No blood | Slightly bloody | Very bloody | |
| *Gills* | | | | |
| Colour | Characteristic<br>Slightly faded | Slightly dark<br>Very faded | Very dark | |
| Mucus | Absent | Moderate | Excessive | |
| Smell | Fresh oily, metallic, seaweed | Fishy | Stale | Spoilt |
| *Belly cavity* | | | | |
| Stains | Opalescent | Greyish | Yellow-brown | |
| Blood | Red | Dark red | Brown | |
| *Total demerit points*[a] | | | | |

[a] Sum of score is from 0 to 27.

represents putrid or spoiled fish. Fish were assessed for odour, flavour and texture. Mean values obtained from assessment of odour, flavour and texture were calculated. To prepare the cooked fish sample, one fish from each storage conditions (in ice and MAP/ice) was filleted and cooked in a microwave oven for 2 min at medium power (650 W). The cooked samples were served hot to panellists for analysis.

## 2.4. Chemical analysis

### 2.4.1. K value

ATP and its degradation products were analysed by using a rapid HPLC method (Özogul, Taylor, Quantick, & Özogul, 2000). The extraction procedure was based on that of Ryder (1985). *K* value was calculated by the procedures described by Saito, Arai, and Matsuyoshi (1959). In this paper, the *K*-value was expressed as a percentage and the formula used is as follows:

$$K\text{-value } (\%) = [(\mathrm{Hx} + \mathrm{INO})/(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{Hx} + \mathrm{INO})] \times 100,$$

where: ATP, Adenosine triphosphate; ADP, Adenosine diphospahte; AMP, Adenosine monophosphate; IMP, Inosine monophosphate; INO, Inosine; Hx, Hypoxanthine.

### 2.4.2. Apparatus for K value

A high-performance liquid chromatography (HPLC) system with an intelligent pump (Merck–Hitachi L-6200A) and a diode array detector (Merck–Hitachi L-4500) was used. The separations were performed on a Sphereclone ODS 2 $C_{18}$, $150 \times 4.60$ mm, 5 μm particle diameter column (Phenomenex, Macclesfield, Cheshire, UK), with a matching guard cartridge.

The mobile phase consisted of acetonitrile (Philip Harris Scientific, Lichfield, Staffordshire, UK) and phosphate buffer solutions. The injection volume was 5 μl and detection was monitored at 254 nm.

### 2.4.3. Biogenic amines

Biogenic amines were analysed by using an HPLC method (Özogul, Taylor, Quantick, & Özogul, 2002). Benzoyl chloride as a derivatization reagent was used and derivatization procedure was based on that of Redmond and Tseng (1979).

### 2.4.4. Apparatus for biogenic amines

A high-performance liquid chromatography (HPLC) system (LaChrome, Merck-Hitachi, Merck Ltd., Poole, Dorset, UK) consisted of an interface (Model D-70000) equipped with a diode array detector (Model L-7455), a pump (Model L-7100), a column oven (Model L-7300), and an auto sampler (Model L-7200). LaChrome (Merck-Hitachi) D-7000 chromatography data station software was used for data acquisition and instrument control. The column, purchased from Phenomenex (Macclesfield, Cheshire, UK) was a Columbus C18 ($150 \times 4.6$ mm).

### 2.4.5. Reagents

Biogenic amine standards (putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihyrochloride, spermine tetrahydrochloride, histamine dihydrochloride, tyramine hydrochloride, and agmatine sulphate) were purchased from Sigma–Aldrich, Poole,

Dorset, UK. The mobile phase consisted of acetonitrile and HPLC grade water (Philip Harris Scientific, Lichfield, Staffordshire, UK).

### 2.5. Microbiological analysis

One sample, from each of three different fish (triplicate) stored under the two different storage conditions for each treatment, was taken to estimate total viable counts. Ten grammes of fish muscle were mixed with 90 ml of Ringer solution and then stomached for 3 min. Further decimal dilutions were made up to $10^{-6}$ and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar plates, in triplicate. They were then incubated for 2 days at 30 °C.

### 2.6. Statistical analysis

Analyses were run in triplicate for each replicate. Data were subjected to a one-way ANOVA (analysis of variance) at a 95% level of significance. Statistical significance is indicated for $P < 0.05$.

## 3. Results and discussion

### 3.1. Sensory analysis

Table 3 shows freshness scores obtained from rainbow trout stored in ice and MAP/ice from day 0 to day 17. Demerit points increased in both conditions up to 17 days with a higher increase for the fish stored in MAP/ice. Demerit points obtained from fish in MAP/ice increased faster after 7 days of storage, which was due to increased drip in MAP packing. However, there were no significant differences ($P > 0.05$) in demerit points between fish in ice and fish in MAP/ice throughout the 17-day storage period. According to the test panel, the acceptable shelf life was found to correspond with a demerit score of 10.53 ± (standard deviation) 0.18 for ice (after 14 days of storage) and 11.38 ± .0.22 for MAP condition (after 10 days of storage).

ANOVA of data within a storage time and treatment showed no effect of diet on sensory scores. However, sensory assessment of rainbow trout fed the experimental diets revealed differences during the different storage conditions (ice and MAP/ice). The rate of increase in demerit points in fish in MAP/ice was higher than that in fish in ice. This was due to the higher demerit points assigned by the panellists to the parameters related to skin, appearance of slime and odour of fish. On the whole the appearance score increased with time, indicating the progressive loss of freshness in both ice and MAP/ice storage. However, the appearance of rainbow trout was poorer in MAP/ice than in ice alone, due to the in-

Table 3
The sensory analysis of rainbow trout stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 | 2.0 ± 0 |
| 3 | 3.85 ± 0.35 | 3.5 ± 0.11 | 3.5 ± 0.21 | 3.8 ± 0.21 | 3.8 ± 0.11 | 4.63 ± 0.18 | 4.5 ± 0.07 | 4.6 ± 0.18 | 4.7 ± 0.07 | 4.8 ± 0.14 |
| 7 | 7.10 ± 0.14 | 7.4 ± 0 | 7.2 ± 0.07 | 7.3 ± 0 | 7.2 ± 0.32 | 8.10 ± 0.14 | 8.1 ± 0 | 8.1 ± 0.49 | 8.3 ± 0.14 | 8.5 ± 0.28 |
| 10 | 9.25 ± 0.21 | 9.2 ± 0.18 | 9.0 ± 0.32 | 9.0 ± 0 | 9 ± 0.18 | 11.40 ± 0.57 | 11.5 ± 0.35 | 11 ± 0.14 | 11.5 ± 0 | 11.5 ± 0.35 |
| 14 | 10.68 ± 0.11 | 10.5 ± 0 | 10.25 ± 0.28 | 10.7 ± 0.18 | 10.5 ± 0.14 | 13.50 ± 0.07 | 13.5 ± 0.18 | 13.25 ± 0 | 14.2 ± 0.14 | 14 ± 0.53 |
| 17 | 20 ± 0 | 20.4 ± 0.28 | 20 ± 0 | 20.4 ± 0.42 | 20.6 ± 0.52 | 24.40 ± 0.28 | 25 ± 0 | 24.6 ± 0.14 | 24.8 ± 0.28 | 24.4 ± 0.14 |

Table 4
The flavour score of cooked rainbow trout associated with odour, flavour and texture

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 9.08 ± 0.01 | 9.07 ± 0 | 9.07 ± 0.07 | 9.05 ± 0.04 | 9.15 ± 0.07 | 8.91 ± 0.07 | 8.99 ± 0.14 | 8.93 ± 0.07 | 8.61 ± 0.21 | 8.71 ± 0.07 |
| 3 | 8.84 ± 0.01 | 8.62 ± 0.14 | 8.42 ± 0.14 | 8.53 ± 0.07 | 8.33 ± 0.14 | 9.27 ± 0.14 | 8.02 ± 0.08 | 7.92 ± 0 | 8.53 ± 0 | 8.33 ± 0.02 |
| 7 | 8.04 ± 0.06 | 7.75 ± 0 | 7.75 ± 0.02 | 8.11 ± 0.07 | 8.08 ± 0 | 7.88 ± 0.08 | 7.65 ± 0 | 7.75 ± 0.08 | 7.75 ± 0.06 | 7.75 ± 0.01 |
| 10 | 6.27 ± 0.14 | 6.57 ± 0.07 | 6.67 ± 0.14 | 6.53 ± 0.21 | 6.33 ± 0.07 | 6.17 ± 0 | 6.03 ± 0.07 | 6.33 ± 0.01 | 5.93 ± 0.01 | 5.83 ± 0 |
| 14 | 5.58 ± 0.11 | 5.43 ± 0.02 | 5.40 ± 0.06 | 5.58 ± 0.02 | 5.50 ± 0 | 5.70 ± 0 | 5.63 ± 0.08 | 5.60 ± 0.11 | 5.40 ± 0.08 | 5.40 ± 0.22 |
| 17 | 3.68 ± 0.07 | 3.50 ± 0 | 3.5 ± 0.07 | 3.80 ± 0.07 | 3.7 ± 0.08 | 3.55 ± 0.07 | 3.50 ± 0.01 | 3.4 ± 0.04 | 3.60 ± 0.14 | 3.48 ± 0.01 |

creased drip. Davis (1998, chap. 9) indicated that the $CO_2$ solubility could alter the food–water-holding capacity and thus increase drip.

Rodríguez, Besteiro, and Pascual (1999) found that gutted rainbow trout stored in ice were acceptable for 6 days whereas Vaz-Pires, Araujo, and Kirby (1995) reported that organoleptic rejection of the ungutted rainbow trout was reached at 11 days in ice storage. However, in this study, the shelf life of gutted rainbow trout, as determined by panellists for fish stored in ice, was still acceptable after 14 days, whereas fish in MAP/ice were not acceptable at 14 days of storage. This long shelf life of fish was achieved due to the maintenance of chilled temperature at harvest and during storage, good hygiene and handling practices. This result is in agreement with those of Dawood, Roy, and Williams (1986) who studied the effect of delaying icing on the storage life of rainbow trout. They found that fish iced immediately after delivery were acceptable, even after 14 days of iced storage in relation to sensory analysis.

A sensory quality test was carried out, in parallel, to establish the rejection point of the cooked rainbow trout fillets. Table 4 shows sensory evaluation scores of rainbow trout fillets. After cooking, all samples were rated as of similar quality. The reference samples did not significantly differ ($P > 0.05$) from other samples in mean scores. The sensory score for flavour of the cooked fillets decreased with storage time. The fresh flavour characteristics of the species were strong between 0 and 7 days, slowly decreasing in intensity to the flavourless stage by 10–14 days. Off-flavour was detected after 14 days due to bacterial metabolites. As spoilage progressed, the off-flavour increased in intensity until the fish were no longer edible by 17 days. The rejection point for the cooked fillets was below 4 at 17 days.

### 3.2. Chemical analysis

#### 3.2.1. ATP-related compounds

Changes in the concentrations of nucleotides and related compounds on storage were similar for the other diets (data are not shown). The main changes are the loss of IMP and the increase in Hx and INO with storage. ATP, ADP, and AMP levels were very low at the beginning of the storage period and then decreased rapidly over the first 3 days of the storage period for fish stored in both ice and MAP/ice. The metabolism of IMP, and formation of INO and Hx appeared to be more rapid in ice than in MAP/ice.

The concentrations of IMP in fresh fish fed diets 4 and 5 were somewhat lower (>8 μmol/g) than in fresh fish fed diets 1, 2 and 3 (>11 μmol/g). IMP was the predominant nucleotide initially present and it was steadily degraded to inosine over 17 days, for both storage conditions. As a result of this, inosine concentration increased until 10–14 days and decreased afterwards to the end of the

17 days of ice storage. IMP is strongly associated with flavour intensity and acceptability in fish (Bremner, Olley, Stratham, & Vail, 1988). Atlantic salmon and rainbow trout produce, predominantly, inosine and therefore inosine level could be used as an indication of the rate of post-mortem degradation (Erikson, Beyer, & Sigholt, 1997; Thomas, Pankhurst, & Bremner, 1999).

Hypoxanthine (Hx) has been reported to be an index of freshness of fish, but the accumulation of Hx varies, both within given species and between species (Huss, 1988). The concentration of Hx varies, even between light and dark muscle. Murata and Sakaguchi (1986) reported that the Hx level is much higher in dark muscle than light muscle. In this study, Hx concentration increased with the increase of storage period, in agreement with what has been reported previously (Boyle, Lindsay, & Stuiber, 1991; Dawood et al., 1986; Greene, Babbitt, & Reond, 1990; Hughes & Jones, 1966; Kyrana, Lougovois, & Valsamis, 1997; Murata & Sakaguchi, 1986; Price, Melvin, & Bell, 1991). The levels of Hx in fish stored in ice were similar to those previously reported for different fish species (Azam, Mackie, & Smith, 1989; Thomas et al., 1999). However, hypoxanthine values, observed for aerobically held fish samples, were higher than those for fillets held in a carbon dioxide modified atmosphere. Carbon dioxide appeared to inhibit formation of hypoxanthine. This is in agreement with previous studies (Boyle et al., 1991; Handumrongkul & Silva, 1994; Warthesen, Waletzko, & Butsa, 1980). However, Brown et al. (1980)found that hypoxanthine values varied widely, with no particular effect due to modified atmospheres for rockfish fillets and silver salmon steaks held in atmospheres containing 20% or 40% $CO_2$.

The concentrations of ATP and its breakdown products are considered to be the most reliable and useful indicators for the measurement of fish freshness (Karube, Matsuoka, Suzuki, Watanabe, & Toyama, 1984). Although the pattern of ATP breakdown varies between species, it has been used to determine freshness in a variety of fish when expressed as the K value (Handumrongkul & Silva, 1994; Lin & Morrissey, 1994; Ryder, Buisson, Scott, & Fletcher, 1984; Vazquez-Ortiz, Pacheco-Aguilar, Logo-Sanchez, & Villegas-Ozuna, 1997). *K*-values were generally low for all treatments (Table 5) for the fresh fish (day zero). However, two exceptional groups of fish fed diets 4 and 5 were plotted as these fresh fish showed somewhat higher *K*-values (15%, 17%) than the other fish (12%). These values of *K* appear to be lower than those reported by other workers (Azam et al., 1989; Azam, Mackie, & Smith, 1990; Thomas et al., 1999). The low *K*-values (<20%) reflected the effects of inhibiting enzyme activity by immediate icing of the fish at harvest. *K* values of trout increased gradually with increase in storage time. However, fish in ice produced higher *K* values during the storage per-

Table 5
The *K* values of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 13.0 ± 0.60 | 12.8 ± 0.92 | 11.5 ± 0.56 | 15.4 ± 0.96 | 17.8 ± 0.43 | 12.2 ± 0.93 | 12.5 ± 0.20 | 11.2 ± 0.94 | 15.2 ± 0.80 | 16.2 ± 0.75 |
| 3 | 21.7 ± 0.74 | 23.3 ± 1.68 | 21.0 ± 1.15 | 27.1 ± 1.44 | 26.5 ± 0.91 | 19.4 ± 1.10 | 20.9 ± 1.10 | 18.2 ± 1.91 | 21.7 ± 2.44 | 20.7 ± 0.71 |
| 7 | 47.6 ± 0.21 | 47.2 ± 1.27 | 45.3 ± 0.43 | 52.0 ± 1.15 | 49.9 ± 1.18 | 39.0 ± 0.80 | 37.4 ± 2.76 | 38.2 ± 0.56 | 44.3 ± 2.80 | 42.5 ± 1.27 |
| 10 | 59.4 ± 0.67 | 58.8 ± 2.77 | 57.0 ± 1.40 | 63.1 ± 2.20 | 64.7 ± 2.4 | 53.1 ± 0.45 | 54.2 ± 0.13 | 51.2 ± 2.13 | 59.3 ± 3.31 | 57.6 ± 2.32 |
| 14 | 73.2 ± 0.19 | 74.5 ± 3.30 | 72.2 ± 2.07 | 78.1 ± 3.1 | 77.3 ± 0.41 | 67.6 ± 1.76 | 67.8 ± 0.76 | 68.1 ± 2.79 | 71.0 ± 0.13 | 71.5 ± 2.69 |
| 17 | 86.9 ± 0.39 | 86.8 ± 1.15 | 85.0 ± 2.19 | 92.5 ± 3.30 | 89.1 ± 0.15 | 81.0 ± 2.21 | 83.8 ± 1.98 | 79.8 ± 0.23 | 87.7 ± 1.12 | 83.9 ± 1.20 |

iod of 17 days than fish in MAP/ice, although statistical analysis (ANOVA) of $K$ values in that storage period indicated no significant differences ($P > 0.05$) between MAP/ice storage and ice storage. Boyle et al. (1991) studied the effects of a carbon dioxide modified atmosphere on the degradation of adenine nucleotides in chill-stored whitefish and rainbow trout during storage for up to 26 days. They indicated that a carbon dioxide atmosphere did not alter $K$ values compared to those observed for aerobically held fish. However, carbon dioxide caused a reduction in the concentration of hypoxanthine compared to aerobically held samples. López-Galvez, de la Hoz, Blanco, and Ordonez (1998) also reported no effect of the storage atmosphere on the $K$ values of sole fillets. Although the trout in MAP/ice showed shorter shelf life with reference to sensory analysis, $K$ value was lower in trout stored in MAP/ice than trout in ice. There was an increase in $K$ value with an increase in storage time, suggesting that $K$ value provided an useful indicator for freshness in rainbow trout stored in both ice and MAP/ice. Similar results were obtained by Luong, Male, and Huynh (1991), Huynh, Mackey, and Gawley (1992) and Malle and Pezennec (1992).

### 3.2.2. Biogenic amines

The concentrations of the biogenic amines in the muscle of rainbow trout kept for 17 days storage in ice, and MAP/ice are given in Tables 6–11 Among the biogenic amines, histamine is associated with scombero-toxicosis (Arnold & Brown, 1978; Lehane & Olley, 2000). In the present study, although trout contained the amino acid histidine, histamine, produced by bacterial decarboxylation of free histidine (Fernández-Salguero & Mackie, 1979) was not detected in any of samples. This result is in agreement with those of Yamanaka, Shiomi, and Kikuchi (1989) and Rodríguez et al. (1999). However, Dawood, Karkalas, Roy, and Williams (1988) reported that histamine concentration increased (<1–4.5 μg/g) during chilled storage of gutted rainbow trout at 0 °C but was lower than the control limit (5 μg/g) fish-legal limit for histamine set by the US Food and Drug Administration (FDA, 1996).

Putresine is produced by either decarboxylation of ornithine or agmatine. The putrescine levels in present study were lower than have been reported in other investigations of spoilage of rainbow trout (Dawood et al., 1988; Rodríguez et al., 1999). The low level of putrescine in gutted fish may be related to the diminished enzymic activity due to the elimination of the contaminating microflora (Dawood et al., 1988).

Cadaverine is produced by decarboxylation of lysine. There was no significant difference ($P > 0.05$) in cadaverine levels between the control diet in ice or MAP/ice and the other experimental diets, throughout in ice, or MAP/ice, during the storage period. The cadaverine

Table 6
Putresine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.21 ± 0.03 | 0.13 ± 0 | 0.26 ± 0.05 | 0.38 ± 0.06 | 0.41 ± 0.03 | 0.35 ± 0.02 | 0.18 ± 0.02 | 0.30 ± 0.02 | 0.41 ± 0.03 | 0.39 ± 0.05 |
| 3 | 1.13 ± 0.01 | 1.28 ± 0.02 | 1.47 ± 0.6 | 1.30 ± 0.03 | 1.45 ± 0.0.3 | 0.83 ± 0.01 | 1.05 ± 0.01 | 1.35 ± 0.03 | 1.45 ± 0.0.3 | 1.56 ± 0.9 |
| 7 | 3.40 ± 0.6 | 3.20 ± 1.2 | 3.29 ± 2.1 | 3.34 ± 1.1 | 2.96 ± 1.8 | 2.17 ± 1.2 | 2 ± 0.4 | 2.71 ± 0.5 | 2.96 ± 1.8 | 2.07 ± 0.04 |
| 10 | 5.30 ± 0.9 | 5.62 ± 0.7 | 4.49 ± 0.4 | 4.30 ± 2.5 | 3.53 ± 2.2 | 3.11 ± 0.8 | 4.20 ± 0.6 | 4.40 ± 2.2 | 3.53 ± 2.2 | 4.28 ± 1.6 |
| 14 | 6.70 ± 1.6 | 6.80 ± 0.8 | 6.88 ± 1.5 | 5.65 ± 1.8 | 4.15 ± 1.9 | 5.20 ± 1.5 | 5.91 ± 2.2 | 5.53 ± 1.7 | 4.15 ± 1.9 | 5.27 ± 1.1 |
| 17 | 8.78 ± 1.3 | 9.96 ± 1.6 | 8.63 ± 2.2 | 9.60 ± 1.2 | 7.40 ± 1.3 | 7.20 ± 1.1 | 8.41 ± 1.3 | 7.60 ± 1.3 | 7.40 ± 1.3 | 8.10 ± 0.9 |

Table 7
Cadaverine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | – | – | – | 0.36 ± 0.06 | – | – | 0.26 ± 0.03 | – | – | – |
| 3 | – | 0.45 ± 0.08 | 0.38 ± 0.05 | 0.52 ± 0.05 | 0.26 ± 0.02 | 0.28 ± 0.04 | – | 0.15 ± 0.01 | 0.68 ± 0.08 | – |
| 7 | 0.35 ± 0.3 | – | 0.66 ± 0.01 | 0.78 ± 0.02 | 0.54 ± 0.01 | – | 0.49 ± 0.01 | 0.45 ± 0.08 | – | 0.35 ± 0.04 |
| 10 | 0.60 ± 0.5 | 0.90 ± 0.01 | 0.88 ± 0.02 | 1.11 ± 0.02 | 1.10 ± 0.02 | 0.75 ± 0.6 | 1.27 ± 0.02 | – | 1.08 ± 0.7 | 0.69 ± 0.02 |
| 14 | 1.50 ± 1.2 | 2.50 ± 2.2 | 2.02 ± 0.4 | 3.13 ± 0.4 | 2.15 ± 0.05 | 1.38 ± 1.1 | 1.72 ± 1.3 | 1.03 ± 0.4 | 2.91 ± 0.9 | 1.03 ± 0.02 |
| 17 | 3.80 ± 0.9 | 4.10 ± 2.5 | 4.54 ± 1.2 | 4.18 ± 0.9 | 4.62 ± 1.6 | 3.97 ± 1.5 | 3.80 ± 0.6 | 3.13 ± 1.2 | 2.98 ± 1.7 | 3.85 ± 1.5 |

Table 8
Spermidine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.96 ± 0.02 | 1.32 ± 0.03 | 1.50 ± 1.2 | 1.43 ± 0.03 | 1.41 ± 0.03 | 1.20 ± 0.02 | 1.45 ± 0.03 | 1.63 ± 0.9 | 1.36 ± 0.5 | 1.55 ± 0.4 |
| 3 | 2.40 ± 1.5 | 2.87 ± 1.3 | 2.60 ± 2.3 | 2.73 ± 0.9 | 2.65 ± 0.6 | 1.97 ± 0.4 | 2.19 ± 0.5 | 1.91 ± 0.05 | 2.69 ± 2.1 | 2.56 ± 2.3 |
| 7 | 3 ± 0.4 | 3.47 ± 0.4 | 3.38 ± 1.7 | 3.48 ± 2.3 | 3.54 ± 1.4 | 2.50 ± 2.2 | 3.14 ± 1.6 | 2.91 ± 1.2 | 2.87 ± 2.6 | 3.16 ± 1.4 |
| 10 | 6.10 ± 0.8 | 5.76 ± 1.3 | 5.68 ± 1.2 | 5.49 ± 1.2 | 5.65 ± 2.2 | 4.80 ± 1.4 | 4.35 ± 0.4 | 4.48 ± 2.5 | 4.79 ± 1.5 | 4.58 ± 0.9 |
| 14 | 3.70 ± 1.8 | 4.98 ± 1.5 | 4.66 ± 0.4 | 4.57 ± 0.6 | 4.63 ± 0.9 | 5.21 ± 0.9 | 5.73 ± 0.8 | 5.86 ± 1.7 | 5.57 ± 1.1 | 5.18 ± 2.4 |
| 17 | 3.21 ± 1.9 | 3.38 ± 1.2 | 3.60 ± 1.1 | 3.80 ± 1 | 3.91 ± 1.2 | 3.50 ± 0.8 | 4.25 ± 1.5 | 3.69 ± 0.9 | 3.45 ± 2.3 | 5.68 ± 2.2 |

Table 9
Spermine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2.10 ± 1.1 | 3.10 ± 2.4 | 2.83 ± 0.5 | 2.82 ± 1.4 | 2.13 ± 0.05 | 2.45 ± 0.6 | 3.41 ± 0.7 | 2.75 ± 1.2 | 3.05 ± 2.2 | 2.23 ± 1.5 |
| 3 | 1.20 ± 0.4 | 1.71 ± 0.5 | 1.92 ± 0.4 | 1.14 ± 0.8 | 1.02 ± 0.02 | 1.45 ± 1.3 | 1.81 ± 0.04 | 1.80 ± 1.3 | 1.94 ± 0.9 | 0.89 ± 0.02 |
| 7 | 3.98 ± 0.2 | 3.19 ± 1.6 | 3.59 ± 2.2 | 3.99 ± 1.2 | 3.56 ± 2.6 | 3.20 ± 1.2 | 2.79 ± 0.5 | 2.57 ± 1.5 | 3.58 ± 1.4 | 3.18 ± 0.6 |
| 10 | 4.10 ± 1.4 | 4.05 ± 1.1 | 4.44 ± 1.3 | 4.51 ± 2.4 | 4.49 ± 1.3 | 3.75 ± 2.3 | 3.90 ± 1.3 | 3.60 ± 0.7 | 4.03 ± 1.7 | 3.87 ± 2.2 |
| 14 | 5.10 ± 1.3 | 5.37 ± 0.9 | 5.69 ± 2.3 | 5.85 ± 0.6 | 5.68 ± 0.7 | 5 ± 1.7 | 4.80 ± 0.9 | 4.89 ± 1.6 | 5.05 ± 1.3 | 5.19 ± 1.8 |
| 17 | 4.36 ± 1.7 | 3.98 ± 1.7 | 3.80 ± 0.5 | 4.12 ± 2.3 | 4.20 ± 2.2 | 4.10 ± 1.3 | 5.10 ± 1.4 | 5.56 ± 2.1 | 5.75 ± 0.7 | 5.56 ± 0.7 |

Table 10
Agmatine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | – | – | – | – | – | – | – | – | – | – |
| 3 | – | – | – | – | – | – | – | – | – | – |
| 7 | – | – | – | – | – | – | – | – | – | – |
| 10 | – | – | – | – | – | – | – | – | – | – |
| 14 | 3.03 ± 1.7 | 3.50 ± 0.4 | 3.20 ± 1.3 | 3.12 ± 0.9 | 3.25 ± 0.8 | 1.65 ± 1.2 | 1.87 ± 1.4 | 1.05 ± 0.4 | – | – |
| 17 | 2.47 ± 1.5 | 2.50 ± 0.6 | 1.25 ± 0.02 | 1.80 ± 1.6 | 1.10 ± 0.02 | 1.80 ± 0.2 | 2.10 ± 0.05 | 2.30 ± 2.2 | 1.24 ± 0.5 | 1.37 ± 0.8 |

Table 11
Tyramine concentrations (mg/100 g) of rainbow trout fed experimental diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | – | – | – | – | – | – | – | – | – | – |
| 3 | – | – | – | – | – | – | – | – | – | – |
| 7 | – | – | – | – | – | – | – | – | – | – |
| 10 | – | 0.99 ± 0.4 | 0.64 ± 0.09 | – | 0.71 ± 0.01 | – | – | – | – | |
| 14 | 0.74 ± 0.08 | 1.31 ± 0.5 | 1.29 ± 0.03 | 1.10 ± 0.02 | 2.06 ± 0.7 | – | 1.75 ± 1.4 | 1.38 ± 1 | – | 0.68 ± 0.08 |
| 17 | 1.54 ± 0.03 | 2.47 ± 0.4 | 2.69 ± 0.8 | 2.37 ± 0.6 | 2.60 ± 1.3 | 0.90 ± 0.8 | 1.07 ± 0.02 | 0.85 ± 0.5 | 1.39 ± 0.04 | 1.35 ± 1.2 |

Table 12
TVC (Log 10 $CFUg^{-1}$) in rainbow trout fed different diets and stored in ice and MAP/ice

| Storage days | Diet 1-ice | Diet 2-ice | Diet 3-ice | Diet 4-ice | Diet 5-ice | Diet 1 MAP/ice | Diet 2 MAP/ice | Diet 3 MAP/ice | Diet 4 MAP/ice | Diet 5 MAP/ice |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 3.18 ± 0.12 | 3.15 ± 0.11 | 3.11 ± 0.24 | 3.13 ± 0.02 | 3.16 ± 0.06 | 3.04 ± 0.13 | 3.17 ± 0.23 | 3.11 ± 0.08 | 3.15 ± 0.18 | 3.18 ± 0.02 |
| 3 | 4.64 ± 0.14 | 4.7 ± 0.06 | 4.66 ± 0.26 | 4.65 ± 0.08 | 4.72 ± 0.05 | 4.49 ± 0.05 | 4.54 ± 0.17 | 4.35 ± 0.12 | 4.50 ± 0.17 | 4.43 ± 0.24 |
| 7 | 5.26 ± 0.17 | 5.28 ± 0.15 | 5.30 ± 0.04 | 5.41 ± 0.20 | 5.38 ± 0.15 | 5.05 ± 0.10 | 4.96 ± 0.08 | 4.91 ± 0.28 | 4.96 ± 0.04 | 4.89 ± 0.15 |
| 10 | 5.68 ± 0.20 | 5.75 ± 0.09 | 5.62 ± 0.14 | 5.73 ± 0.10 | 5.65 ± 0.04 | 5.14 ± 0.18 | 5.30 ± 0.04 | 5.22 ± 0.13 | 5.16 ± 0.02 | 5.39 ± 0.12 |
| 14 | 5.96 ± 0.15 | 5.88 ± 0.22 | 5.92 ± 0.17 | 5.95 ± 0.18 | 5.90 ± 0.18 | 5.44 ± 0.23 | 5.60 ± 0.19 | 5.56 ± 0.19 | 5.45 ± 0.29 | 5.72 ± 0.26 |
| 17 | 6.70 ± 0.18 | 6.95 ± 0.04 | 6.81 ± 0.21 | 6.92 ± 0.15 | 7.02 ± 0.22 | 6.04 ± 0.11 | 6.21 ± 0.28 | 6.23 ± 0.05 | 6.31 ± 0.17 | 6.39 ± 0.13 |

concentration increased, during 17 days of storage of trout, in both ice and MAP/ice (Tables 6–12). However, cadaverine levels in the present study were higher than those reported in studies of spoilage in rainbow trout (Rodríguez et al., 1999; Dawood et al., 1988) and lower than those of spoilage of seawater fish (Duflos, Devrin, Malle, & Bouquelet, 1999; Ritchie & Mackie, 1980; Wendakoon, Murata, & Sakaguchi, 1990). Yamanaka et al. (1989) suggested that cadaverine can be used as an index of freshness in salmonids with acceptability limit of about 10 mg/100 g fish. However, in the present study, the concentration of cadaverine in samples that were not acceptable at 17 days of storage, in both ice and MAP/ice conditions, in terms of sensory analysis, were much lower than this value.

Agmatine and tyramine were not detected in the samples until 14 or 17 days and 10 or 14 days for ice storage, respectively, and until 14 or 17 days for MAP/ice storage. This is in agreement with the observation of Rodríguez et al. (1999) who reported that these two amines were not detected in trout stored in ice after gutting except for small amounts in trout stored under refrigeration after gutting and vacuum-packing. Dawood et al. (1988), also, did not detect tyramine during storage of chilled rainbow trout.

Spermine and spermidine are usually the major amines present in fresh muscle and their concentrations depend upon the species of fish, the free amino acids present in the tissue and the conditions of exposure to spoilage bacteria (Duflos et al., 1999). The lowest concentration of spermine in fresh fish was obtained from trout fed diet 1 (2.10 mg/100 g) for ice storage and from trout fed diet 5 (2.23 mg/100 g) for MAP/ice storage. Statistical analysis showed no significant difference ($P > 0.05$) in spermine and spermidine concentrations in fish fed the experimental diets compared to the fish fed the control diet, for both storage conditions. Mieltz and Karmas (1978) found that spermidine and spermine levels decreased as decomposition of salmon steaks progressed while putrescine, cadaverine and histamine increased.

Although there were no significant differences ($P > 0.05$) in the concentration of biogenic amines in fish fed experimental diets containing different plant proteins as a result of storage in ice alone or in MAP/ice, lower concentrations of amines were found in the samples of fish in MAP than in the samples of fish in ice in the present study. This is supported by Özogul, Taylor, Quantick, and Özogul (2002b) who studied changes in biogenic amines in herring stored under modified atmosphere (60% $CO_2$ and 40% $N_2$) and under vacuum. They found that the concentrations of biogenic amines in herring stored in MAP were lower than those stored in ice and vacuum pack. In this study, the level of biogenic amines, regardless of the diet treatment, showed a similar trend, indicating that dietary ingredients did not have any effect on the biogenic amine content of fish although lower biogenic amine values were obtained from trout stored in MAP/ice. Since histamine was not detected and the concentration of other amines were low, non-volatile amine production is not very significant, even until upto the 17th day of storage of trout in ice and MAP/ice.

### 3.3. Microbiological analysis

Microbial counts on rainbow trout stored in ice and MAP/ice are shown in Table 12. The initial quality of

fish used in this study, for all diets, was good, as indicated by a low initial number of bacteria ($10^3$ CFU $g^{-1}$) before fish were subjected to the different storage conditions. There was an increase in total viable counts over the period of storage. Bacteria grew more quickly in rainbow trout kept in ice than in MAP/ice. Similar results were reported by Randell, Hattula, and Ahvenainen (1997) for rainbow trout, by Yasuda, Nishino, Chiba, Nakano, and Yokoyama (1989) for yellowtail fillets, by Parkin, Wells, and Brown (1981) for rockfish fillets, by López-Galvez et al. (1998) for sole fillets, by Woyewoda, Bligh, and Shaw (1984) for cod fillets and by Wilhelm (1982) for rockfish, salmon, trout and croaker. It has been also reported that carbon dioxide has been shown to delay spoilage of fresh seafood by inhibiting psychrotopic, aerobic and Gram negative bacteria (Banks et al., 1980; Brown et al., 1980; Finne, 1982). To achieve microbiological benefit, the storage temperature of MAP products should be as low as possible, since solubility of $CO_2$ decreases with increase in temperature (Daniels, Krishnamurth, & Rizvi, 1985; Sivertsvik et al., 2002).

Packing of the rainbow trout in $CO_2$ has been shown to extend the shelf-life in MAP/ice storage. The degree to which the shelf-life is extended depends on the initial number and types of microorganism present on the fish. The presence of carbon dioxide limits microbial growth and changes the types of microorganisms (Banks et al., 1980; Handumrongkul & Silva, 1994; López-Galvez et al., 1998; Sivertsvik et al., 2002; Villemure, Simard, & Picard, 1986). Although there were no significant differences ($P > 0.05$) in total viable count of fish stored in ice and MAP/ice, regardless of different diets given, fish packed in MAP showed reduction in bacterial counts after 3 days compared to fish held in ice. This continued as the storage time progressed. Microbial growth was inhibited by the presence of $CO_2$ in all treatment groups used throughout the study. If $10^6$ microorganisms/g are considered the TVC limit of acceptability, the shelf life of rainbow trout was 14 days for ice and approximately 15–16 days for MAP/ice. This finding indicated that freshness of trout, in relation to sensory analysis, was lost before bacteria count increased significantly in MAP/ice. Statistical analysis using ANOVA showed that the different diets did not have any significant ($P > 0.05$) effect on the microbial count of the fish when compared to the control diet (diet 1) under both storage conditions.

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